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Original Article

Molecular characterisation of *Klebsiella oxytoca* strains isolated from patients with antibiotic-associated diarrhoea

Mohammad Yousef Alikhani [a], Fereshteh Shahcheraghi [b], Sepideh Khodaparast [a], Amir Sasan Mozaffari Nejad [c,d], Mojgan K. Moghadam [e], Sayed Fazlollah Mousavi [b,*]

[a] Department of Microbiology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
[b] Microbiology Research Center & Department of Microbiology, Pasteur Institute of Iran, Tehran, Iran
[c] Department of Microbiology, Osmania University, Hyderabad, Andhra Pradesh (AP), India
[d] Department of Microbiology, Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
[e] Department of Virology, Pasteur Institute of Iran, Tehran, Iran

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ABSTRACT

Background and study aim: Colitis is a common complication after treatment with antibiotics such as β-lactams, quinolones, and aminoglycosides. Recently, *Klebsiella oxytoca* has been implicated in this type of diarrhoea. The prevalence and characterisations of *K. oxytoca* isolated from patients with antibiotic-associated diarrhoea were investigated. The *K. oxytoca* isolates were also tested for cytotoxin production.
Patients and methods: This study was conducted from May 2011 to Dec 2013. Faecal samples were collected from hospitalised patients receiving antibiotic treatment. Initial cultivation was performed on specific media. The clinical isolates were confirmed by polymerase chain reaction (PCR) using the specific *K. oxytoca* polygalacturonase (pehX) gene. The double-disc diffusion test was used to detect extended-spectrum beta-lactamase (ESBL)-producing strains. Tracking of ESBL-encoding genes was performed via PCR. The organism was cultured on Hep-2 cell lines for cytotoxin production.
Results: Out of 331 samples collected from patients, 40 were confirmed molecularly to be clinical isolates of *K. oxytoca*. Fourteen (35%) ESBL-producing strains were isolated using the double-disc diffusion method. Among the molecularly confirmed *K. oxytoca* isolates, seven (17.5%) tested positive for the *bla*SHV gene, 12 (30%) for *bla*TEM, 10 (25%) for *bla*CTX-M, three (7.5%) for *bla*OXA, nine (22.5%) for *bla*CTX-M-15, and seven (17.5%) for *bla*TEM-1. Five (12%) isolates showed cytotoxin activity below 30%, 12 (30%) strains showed moderate cytotoxin activity between 30% and 60%, and 23 (58%) strains showed cytotoxin activity ⩾60%.
Conclusions: The cytotoxin-producing *K. oxytoca* is found to be one of the causes of antibiotic-induced colitis. Discontinuing treatment and allowing normal intestinal flora to be established or prescribing appropriate medication after antibiogram can help patients with antibiotic-induced haemorrhagic colitis in a timely manner.

Introduction

Antibiotic-associated diarrhoea frequently occurs during antibiotic treatment [1]. In some patients, antibiotic treatment causes the selection and overgrowth of toxin-producing bacteria, which in turn causes antibiotic-associated colitis (AAC). Toxin-producing *Clostridium difficile* is a significant bacterium that causes pseudomembranous colitis [2]. It is the main cause of infectious diarrhoea in hospitals and long-term care facilities [3]. However, this bacterium is seen only in 10–20% of cases of colitis. Antibiotic-associated haemorrhagic colitis (AAHC) is another type of colitis not caused by *C. difficile* [4]. AAHC was first reported in 1978 by Toffler and colleagues from Austria [5]. This form of colitis occurs concurrent with or after treatment with beta-lactam antibiotics, cephalosporins, and quinolones as well. Suspected haemorrhagic colitis caused by antibiotics has been found to resolve spontaneously after antibiotic therapy was discontinued. These complications are a result of various mechanisms such as allergic reactions, mucosal ischaemia, and infections of bacterial or viral origin [5,6].

* Corresponding author.
E-mail address: mousavi@pasteur.ac.ir (S.F. Mousavi).

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Recently, AAHC was shown to be caused by cytotoxin-producing *Klebsiella oxytoca*. Unlike the colitis caused by *C. difficile*, this form of colitis often begins with abdominal pain, bloody diarrhoea, and sudden onset requiring hospitalisation [1,7]. *K. oxytoca* can colonise healthy individuals (1.6–9%), and it has been proven to be an opportunistic pathogen of the intestine [2].

Cytotoxins are key to the pathogenesis of *K. oxytoca*. In a patient with AAHC, cytotoxicity varies with different species of bacteria [8,9]. In the majority of patients with AAHC, a significant number ($>10^6$ CFU/ml) of *K. oxytoca* are observed in the stool [6]. Cephalosporin antibiotics are used for the first-line treatment of *K. oxytoca* infections. In the case of resistance to cephalosporins, aminoglycosides (usually gentamicin), and fluoroquinolones (e.g., ciprofloxacin), carbapenems can be used [10]. *Klebsiella* spp. are able to hydrolyse cephalosporins and even carbapenems [11,12]. The production of β-lactamase enzymes is the most important mechanism of resistance to β-lactam antibiotics [13]. Resistance to aminoglycosides occurs via the methylation of 16S ribosomal RNA (rRNA) gene, which reduces its affinity for aminoglycosides. Several studies confirm that most *arm*A methylase genes, with *bla*CTX-M-15 and *bla*TEM-1 genes, are simultaneously transmitted by conjugation to *K. oxytoca* [14]. Studies have also reported the prevalence of extended-spectrum beta-lactamases (ESBLs) such as TEM, SHV, PER, VEB, CTX-M, and OXA in *K. oxytoca* [15,16].

In our region, no comprehensive study has been conducted on *K. oxytoca*, which is known to induce AAC. Hence, the aim of this study was to determine the prevalence, characteristics, and cytotoxin production of *K. oxytoca* isolated from patients with colitis caused by antibiotic therapy.

Patients and methods

Stool samples

A total of 331 stool samples were collected over an 18-month period (from May 2011 to December 2013) from hospitalised patients (Taleghani, Milad, and Shariati) including children and adults (male and female). The protocol was approved by the ethics committee of the Hamadan University of Medical Sciences, Iran. The majority of patients presented with diarrhoea or bloody diarrhoea and a history of β-lactam antibiotic treatment from 1 week to 2 months, for example, penicillin, imipenem, and meropenem; cephalosporins in general such as ceftriaxone and ceftazidime; and aminoglycosides such as gentamicin and amikacin. The patients were divided into four groups: 259 patients receiving antibiotic therapy and having diarrhoea (A^+D^+, 78%), 39 (12%) patients receiving antibiotic therapy but not having diarrhoea (A^+D^-), 30 (9%) patients not receiving antibiotic treatment but having diarrhoea (A^-D^+), and three patients who did not receive antibiotic treatment and did not suffer from diarrhoea (A^-D^-, 1%) [4].

Isolation and identification of bacteria

The stool samples were transferred to Cary Blair transport medium (Merck, Darmstadt, Germany) via swabs. The demographic features of patients such as sex, age, and duration of antibiotic use were recorded in a questionnaire. The transferred stool samples were then cultured on MacConkey agar medium (Merck, Darmstadt, Germany) and incubated at 37 °C for 18 h. All of the samples for identification of *K. oxytoca* were done on Lactose-positive colonies; biochemical tests such as indole production, Simmons' citrate, triple sugar iron (TSI) agar, lysine decarboxylase, ortho-nitrophenyl-β-galactopyranoside (ONPG), and malonate tests were also performed on the samples [17].

DNA extraction

For this purpose, 2 mL of the overnight bacterial culture was centrifuged, and the pellet was resuspended in 620 mL of lysis buffer (10 mM Tris–HCl, 50 mM ethylenediaminetetraacetic acid (EDTA), and 100 mM NaCl; pH 8) containing 1% sodium dodecyl sulphate (SDS) and 0.4 mg/mL of proteinase K (Sigma, USA). The mixture was incubated for 1 h at 56 °C and then for 1 h at 100 °C. An equal volume of phenol/chloroform/isoamyl alcohol (BDH, UK) was added to the mixture and centrifuged at 10,000 rpm for 10 min. The supernatant was added to an equal volume of chloroform. After centrifugation at 10,000 rpm, the top layer was collected, and DNA was precipitated with two volumes of cold isopropanol (Merck, Darmstadt, Germany) at −20 °C for 10 min. The pellet was obtained by centrifugation for 20 min and then washed with 1.5 mL of 70% cold ethanol (Merck, Darmstadt, Germany). Finally, the pellet was resuspended in 100 mL of TE 1× buffer (10 mM Tris–HCl and 1 mM EDTA; pH 8) and 1 μL was used for the polymerase chain reaction (PCR) mixture [18].

K. oxytoca isolates confirmed by PCR assay

The *K. oxytoca* strains isolated by biochemical tests were molecularly confirmed by the PCR assay using the polygalacturonase pehX gene, a species-specific *K. oxytoca* gene (Table 1) [14,19–21].

The *K. oxytoca* ATCC 13182 strains, provided by the Pasteur Institute of Iran, were used as a positive control. The PCR reaction conditions were as follows: initial denaturation at 95 °C for 2 min

Table 1
Primer sequences.

Gene	Primer name	Primer sequences	Size bp	References
SHV	bla_{SHV} R	5′-GCCTTTATCGGCCTTCACTCAAG-3′	868	[19]
	bla_{SHV} F	5′-TTAGCGTTGCCAGTGCTCGATCA-3′		
TEM	bla_{TEM} R	5′-GAGTATTCAACATTTCCGTGTC - 3′	931	[19]
	bla_{TEM} F	5′-TAATCAGTGAGGCACCTATCTC -3′		
CTX-M	bla_{CTX-M} R	5′-ACCGCGATATCGTTGGT-3′	909	[19]
	bla_{CTX-M} F	5′-CGCTTTGCGATGTGCAG-3′		
OXA	bla_{OXA} R	5′-AGTGTGTTTAGAATGGTGATC-3′	846	[19]
	bla_{OXA} F	5′-ACACAATACATATCAACTTCGC-3′		
CTX-M-15	$bla_{CTX-M-15}$ R	5′-ACCGTCGGTGACGATTTTAG-3′	876	[20]
	$bla_{CTX-M-15}$ F	5′-AGAATAAGGAATCCCATGGTT-3′		
TEM-1	bla_{TEM-1} R	5′-CTG ACA GTT ACC AAT GCT TA-3′	858	[20]
	bla_{TEM-1} F	5′-ATG AGT ATT CAA CAT TTC CG-3′		
*arm*A methylase	*arm* A R	5′-GGAGAAGGGAATGGAAGAGA-3′	592	[14]
	arm A F	5′-AGGTTGTTTCCATTTCTGAG-3′		
peh X	*peh* X C	5′-GATACGGAGTATGCCTTTACGGTG-3′	344	[18]
	peh X D	5′-TAGCCTTTATCAAGCGGATACTGG-3′		

followed by 35 cycles of denaturation at 94 °C for 20 s, annealing at 59 °C for 20 s, and 72 °C for 30 s, and extension at 72 °C for 10 min in a thermocycler device (Eppendorf AG 22331). The PCR products were electrophoresed and subsequently stained with ethidium bromide, following which the gel scan image was prepared. The PCR product (344 bp in size) was found to be positive based on measurements of the DNA size marker (Pars Toos Co., Tehran, Iran).

Phenotypic detection of ESBL enzymes

The isolates were tested for ESBL production with the combined disc test (CDT) as per Clinical & Laboratory Standards Institute (CLSI) guidelines. CDT was performed on ceftazidime- and cefotaxime-resistant strains by placing discs of ceftazidime and cefotaxime (30 μg each) at a 20-mm distance from a disc containing ceftazidime/clavulanic acid (CLA) (30/10 μg) and cefotaxime/CLA (30/10 μg), respectively. If the difference in the inhibition zone diameter is $\geqslant$5 mm, these strains are considered to be ESBL producing [22]. For this method, a half McFarland solution of *K. oxytoca* was prepared and cultured on Mueller–Hinton agar plates (Merck, Darmstadt, Germany).

Detection of antibiotic resistance genes

The blaCTX-M, blaTEM, blaSHV, blaCTX-M-15, blaTEM-1, and armA methylase genes were investigated via the PCR assay with specific primers (Table 1). The *Klebsiella pneumoniae* ATCC 7881 and *K. pneumoniae* ATCC 700603 strains were used as quality controls (Pasteur Institute of Iran).

Cytotoxin production assay of K. oxytoca isolates

All molecularly confirmed *K. oxytoca* strains were tested for cytotoxin production with cell culture assays. The cytotoxic effects were assessed by microscopic examination, as described previously [23–25]. In brief, the cytotoxin production in clinical isolates of *K. oxytoca* was investigated by Hep-2 cell culture. The level of cytotoxin produced was qualitatively determined as a percentage of the number of dead or deformed cells in cell culture on Neobar lam (Fig. 1). Considering the effect of the supernatant on the Hep-2 cells, they were classified into three categories of cells which were fusiform in shape, normal and alive [26]. Also, cells were not spindle shaped and had rounded shapes but they were alive, these two forms were in the first group. The second group consisted of dead cells or cellular debris. Living cells were included in groups I and II and also, the cytotoxin effect was compared with control samples. *K. oxytoca* ATCC 13182 was used as control.

Statistical analysis

Statistical analysis was performed using the chi-squared test with SPSS software (version 16). A *P*-value of $\leqslant$0.05 was considered statistically significant.

Results

The prevalence of *K. oxytoca* was found to be 52.5% in children, 25% in men, and 22.5% in women. After biological and biochemical

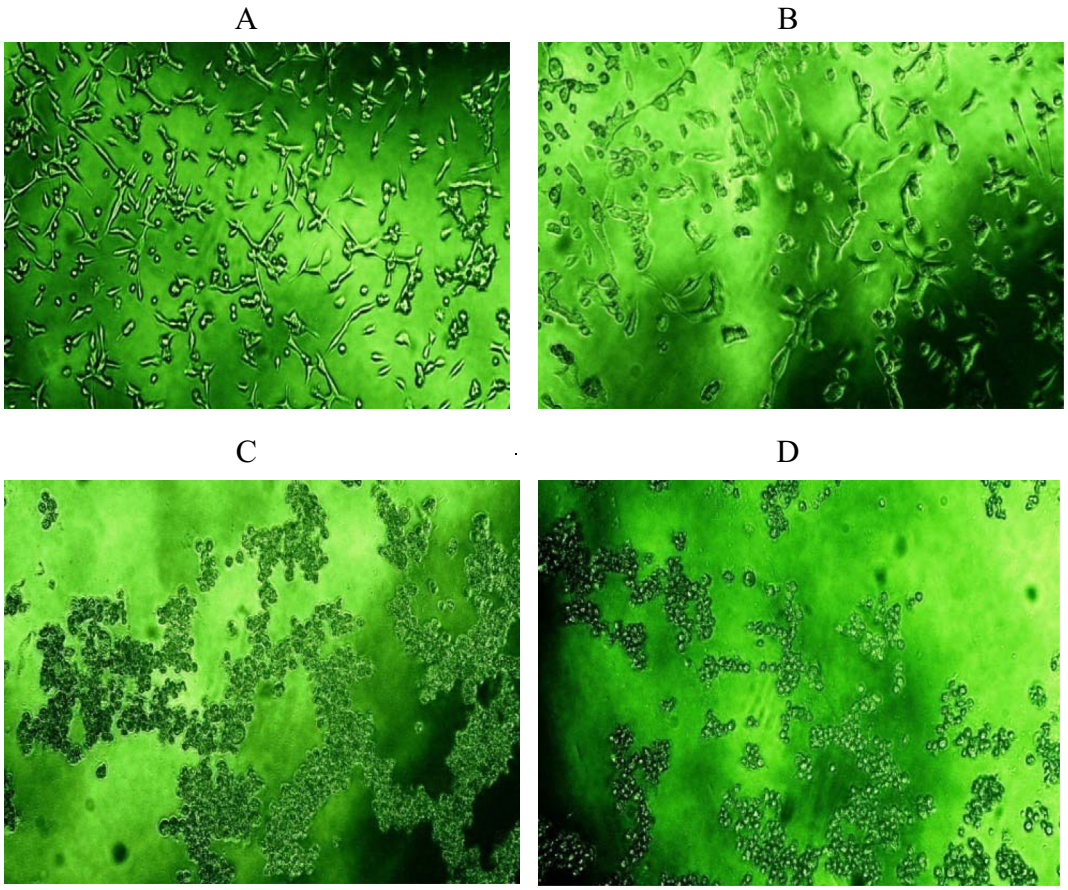

Fig. 1. Cytotoxic effects of bacterial supernatants on Hep-2 cells. (A) Negative control strain supernatants, *Klebsiella oxytoca* ATCC 13182 (failure to cytotoxin production) cells inoculated into Hep-2. Intact cells were seen. (B) Negative control supernatant was diluted 1:1 with PBS. Intact cells were seen. (C) Supernatant samples from patient No. 32. Paediatric patients hospitalised with symptoms of dysentery. Completely dead cells were seen. (D) Supernatant samples from patient No. 32 diluted with PBS 1:1. Crumbling cells were seen.

differentiation, a total of 57 *K. oxytoca* strains were isolated, 40 (12.1%) of which were molecularly confirmed by pehX-specific gene PCR assay. The prevalence of *K. oxytoca* was higher in children (<15 years of age) than in adults (Table 2). Of the 40 patients who were molecularly confirmed to harbour *K. oxytoca*, 36 patients were included in group A^+D^+, one patient in group A^+D^-, three patients in group A^-D^+, and no patients in group A^-D^-. The results of the antibiotic susceptibility pattern showed that the molecularly confirmed clinical isolates of *K. oxytoca* were sensitive to amikacin, ertapenem, imipenem, and meropenem antibiotics (97.5%, 97.5%, 92.5%, and 90%, respectively). The average sensitivity to the cephalosporin group (e.g., cefepime, cefotaxime, ceftazidime, and ceftriaxone) was 72%. The 12 (85.7%) ESBL-producing strains were found to be resistant (minimum inhibitory concentration (MIC) ⩾ 32 μg/ml) to ceftazidime. High resistance rates were observed against amoxicillin (85%), ampicillin (80%), and ticarcillin (55%). The isolated *K. oxytoca* strains showed resistance to three classes of antibiotics: β-lactams, aminoglycosides, and quinolones. A total of 16 *K. oxytoca* isolated strains exhibited multidrug resistance (MDR).

Out of 40 *K. oxytoca* isolated strains, seven (17.5%) were positive for bla_{SHV} gene, 39 (97.5%) for bla_{TEM}, 10 (25%) for $bla_{CTX\text{-}M}$, three (7.5%) for bla_{OXA}, nine (22.5%) for $bla_{CTX\text{-}M\text{-}15}$, and seven (17.5%) for $bla_{TEM\text{-}1}$. The *armA* gene was not found in any of the isolates. In total, six (15%) isolates were positive for both $bla_{CTX\text{-}M\text{-}15}$ and $bla_{TEM\text{-}1}$ genes. *OXA* genes were sequenced, and the type of OXA-1 was confirmed by gene sequencing. The accession numbers are KJ184343, KJ194469, and KJ194470 in GenBank. The prevalence of genes encoding ESBL enzymes in the positive and negative ESBL isolates is listed in Table 3.

Table 2
Results obtained from analysis of *Klebsiella oxytoca* strains isolated from patients.

No.	Age (year)	Sex	Stool app.	Antibiotic therapy	Time of antibiotic therapy (days)	Cytotoxin activity (%)	*peh*X	ESBL	CTX-M	TEM	OXA	SHV	TEM-1	CTX-M-15	*arm*A-methylase
1	31	F	Loose-bloody	Metronidazole	21	20	+	–	–	+	–	+	–	–	–
2	50	F	Watery-brown	Ceftriaxone	7	100	+	–	–	+	–	–	–	–	–
3	1	M	Soft-brown	Metronidazole	1	100	+	–	–	+	–	–	–	–	–
4	3	F	Mucoid-brown	Cefotaxime	6	95	+	–	–	+	–	–	–	–	–
5	5	M	Soft-yellow	Ceftriaxone	1	85	+	–	–	+	–	–	–	–	–
6	2	M	Loose-yellow	Cefixime	1	85	+	–	–	+	–	–	–	–	–
7	45	M	Loose-brown	Metronidazole Imipenem	3 6	90	+	–	–	+	–	–	–	–	–
8	24	M	Loose-bloody	Ciprofloxacin Ceftriaxone Metronidazole	3	50	+	–	–	+	–	–	–	–	–
9	55	F	Loose-brown	Ceftriaxone	7	85	+	–	–	+	–	–	–	–	–
10	33	F	Watery-green	Ciprofloxacin	7	50	+	+	+	+	–	–	+	+	–
11	56	M	Loose-brown	Ceftriaxone Metronidazole	3	75	+	–	–	+	–	–	–	–	–
12	56	M	Loose-brown	Ceftriaxone Metronidazole	3	80	+	–	–	–	–	–	–	–	–
13	63	M	Loose-brown	Metronidazole	7	20	+	–	–	+	–	–	–	–	–
14	50	M	Mucoid-brown	Ceftriaxone Metronidazole	6	20	+	–	–	+	–	–	–	–	–
15	33	F	Soft-brown	Metronidazole	7	20	+	–	–	+	–	–	–	–	–
16	60	F	Loose-brown	Ceftriaxone	7	100	+	+	+	+	–	–	–	–	–
17	2	M	Loose-brown	Ceftriaxone	2	100	+	–	–	+	–	+	–	–	–
18	78	M	Loose-brown	Ceftriaxone	2	50	+	+	+	+	+	–	–	–	–
19	13	M	Loose-brown	–	–	50	+	+	–	+	–	–	–	+	–
20	2	M	Loose-brown	Ciprofloxacin Ceftazidime	2	40	+	–	–	+	–	–	–	–	–
21	1	M	Mucoid-brown	Ceftriaxone	21	30	+	–	–	+	–	–	–	–	–
22	4	F	Loose-brown	Ceftriaxone	2	40	+	–	–	+	–	–	–	–	–
23	1	M	Loose-brown	Ceftriaxone Amikacin	14	30	+	–	–	+	–	+	–	–	–
24	47	M	Form-brown	Ciprofloxacin Amikacin	14	20	+	+	+	+	+	–	–	+	–
25	45	F	Soft-brown	Meropenem Vancomycin	14 2	90	+	+	+	+	+	–	+	+	–
26	40	F	Loose-brown	Ceftriaxone	1	90	+	+	+	+	–	–	+	+	–
27	30	M	Loose-brown	Ceftriaxone	2	80	+	–	–	+	–	–	+	+	–
28	4	M	Loose-brown	Ceftriaxone	1	90	+	–	–	+	–	–	–	–	–
29	1	M	Loose-brown	Ceftazidime Metronidazole	10	100	+	+	+	+	–	–	–	–	–
30	9	F	Watery-brown	Ceftriaxone	14	100	+	+	+	+	–	–	+	+	–
31	<1	M	Loose-yellow	Metronidazole	14	90	+	–	–	+	–	+	–	–	–
32	3	F	Loose bloody	Metronidazole	3	100	+	–	–	+	–	–	–	–	–
33	3	F	Loose bloody	Ceftriaxone	7	60	+	+	+	+	–	–	+	+	–
34	50	M	Loose brown	Ceftriaxone	1	80	+	–	–	+	–	+	–	–	–
35	9	M	Loose brown	–	–	90	+	+	+	+	–	+	–	+	–
36	<1	M	Watery yellow	–	–	50	+	–	–	+	–	+	–	–	–
37	14	F	Loose brown	Ceftriaxone	7	90	+	–	–	+	–	–	–	–	–
38	<1	M	Form brown	Ceftriaxone	1	40	+	+	–	+	–	–	–	–	–
39	<1	M	Loose green	Ceftriaxone	1	50	+	–	–	+	–	–	+	–	–
40	29	F	Loose green	Imipenem Clindamicin	1 1	50	+	–	–	+	–	–	–	–	–
Total								12	10	39	3	7	7	9	0

Table 3
The number of antibiotic resistance genes in strains (ESBLs and non-ESBLs).

	TEM No (%)	CTX-M No (%)	SHV No (%)	OXA No (%)	TEM-1 No (%)	CTX-M-15 No (%)	*arm*A No (%)
ESBLs positive ($n = 12$)	12 (100)	10 (71.4)	1 (7.1)	3 (21.4)	5 (35.7)	8 (57.1)	0 (0)
Non-ESBLs ($n = 28$)	27 (94.4)	0 (0)	6 (23.1)	0 (0)	2 (7.7)	1 (3.8)	0 (0)
Total = 40	39 (97.5)	10 (25)	7 (17.5)	3 (7.5)	7 (17.5)	9 (22.5)	0 (0)

Cytotoxin production assay

The cytotoxin production by clinical isolates of *K. oxytoca* was investigated by Hep-2 cell culture. Five isolated strains (12%) showed cytotoxin activity below 30%. Twelve (30%) strains showed moderate cytotoxin activity between 30% and <60%. Finally, 23 (58%) strains showed cytotoxin activity $\geqslant$60%.

Nevertheless, in the present study, *K. oxytoca* cytotoxin activity was higher in the paediatric population (under 15 years). No significant relationship was found between the age and sex of patients and the cytotoxin activity of isolates. None of the isolates had zero cytotoxin activity, and a low level of activity was seen in a small percentage of isolates (12%), which highlights the significance of the bacterial cytotoxin.

Almost all isolates with moderate to high cytotoxin activity were resistant to ampicillin, ampicillin–sulbactam, cefotaxime, ceftazidime, ciprofloxacin, ticarcillin, and gentamicin. However, isolates with low cytotoxin activity were resistant to a small number of antibiotics, including ampicillin, amoxicillin, and ticarcillin.

Discussion

As *K. oxytoca* plays a key role in antibiotic-associated diarrhoea, which has not been investigated in our area, this study examined the prevalence of these bacteria among hospitalised patients. Out of 331 samples isolated from patients, 40 (12.1%) *K. oxytoca* strains were identified using biochemical and molecular methods. In comparison with other studies, the present results showed a higher prevalence of these bacteria. In previous studies, Zollner-Schwetz et al. in Austria and Gorkiewicz et al. in Nigeria reported the prevalence of *K. oxytoca* in faecal samples collected from patients with haemorrhagic colitis (1.6% and 10%, respectively) [4,6]. A previous study revealed a higher rate of *K. oxytoca* infection in children ($\leqslant$15 years). Therefore, this bacterium can be an important cause of diarrhoea in children [8].

In a previous study on *K. oxytoca* isolates from Spain, 17.3% of strains were resistant to multiple classes of antibiotics (multidrug resistant) [27]. However, the present study reported that 16 (40%) strains were resistant to multiple classes of antibiotics. Although reports on *K. oxytoca* in Iran are scarce, the present study was compared with a previous study by Nasehi et al. from the Pasteur Institute of Iran on *K. pneumoniae*, which did not include faecal samples [28]. Our results were also compared with previous studies on antibiotic-induced *K. oxytoca* colitis in other countries such as Belgium, India, and China [29–31]. The determinations of ESBL-producing strains were compared with other studies [26,32,33].

In this study, the prevalence of ESBLs in *K. oxytoca* isolated strains was approximately 35%, and had an increase the multiplier. ESBL-producing strains showed greater resistance to the cephalosporin group of antibiotics. In addition, the MIC for the ESBL-producing strains to CTX was $\geqslant$16 μg/ml and that for CAZ was $\leqslant$32 μg/ml. Strains with CTX-M genes showed 80% and 90% resistance to ceftazidime and cefotaxime, respectively, as well as 100% resistance to amoxicillin, ampicillin, cefepime, and ticarcillin. A statistically significant correlation was noted between the CTX-M gene and ESBL production. *K. oxytoca* harbouring ESBL-producing genes have not been reported in Iran. In this study, 10 (25%) of the *K. oxytoca* isolates, which were ESBL producing, were found to harbour the *bla*CTX-M gene. β-Lactamase type *bla*CTX-M-15 exerted a catalytic effect against ceftazidime and was inhibited by CLA and tazobactam. Moreover, nine strains harboured *bla*CTX-M-15 and showed 100% resistance to ceftazidime. In addition, the prevalence of SHV genes was 17.5% (seven strains isolated from 40 strains), and only one strain was susceptible to cefepime. Moreover, 97.5% of *K. oxytoca* isolates harboured the *bla*TEM gene; an average of 21% were resistant to the cephalosporin group of antibiotics. Of the ESBL strains, 12 harboured TEM genes. In an Indian study, Sharma et al. reported that PCR performed on plasmid DNA alone detected 30% ESBL-positive isolates using TEM and 38% using SHV primer, whereas PCR for both plasmid and chromosomal DNA showed 56% positivity for TEM and 60% positivity for SHV [34]. Furthermore, Paterson et al. showed that multiple β-lactamases were produced by isolates with phenotypic evidence of ESBL production. Using phenotypic methods, SHV-type ESBLs were found to be the most common ESBL, occurring in 67.1% of isolates. By contrast, TEM-type ESBLs were found in just 16.4% of isolates [35]. The non-ESBL SHV-1 β-lactamase is commonly detected in *Escherichia coli* and *K. pneumoniae*. In addition, the gene is usually plasmid mediated in *E. coli* but chromosomally encoded in most *K. pneumoniae* isolates [36]. Interestingly, in this study, the gene coexisted with $bla_{CTX\text{-}M15}$ in one isolate. However, most isolates expressing the SHV enzyme were non-ESBL producers. Similarly, only eight isolates harboured both $bla_{CTX\text{-}M15}$ and bla_{TEM} genes. The remaining TEM-positive isolates were non-ESBL producers. This finding is not surprising because TEM-1 is the most frequently encountered β-lactamase among ampicillin-resistant *E. coli*. Although these classical β-lactamases are not considered ESBLs, their clinical significance is linked to their ability to undergo mutations to increase their activity against ESBLs (e.g., third-generation cephalosporins) [37].

In this study, the prevalence of the OXA gene was 7.5%. Furthermore, 100% of OXA-positive strains were found to be resistant to cefepime. Among three strains harbouring the OXA gene, two were resistant to imipenem and all were resistant to meropenem.

Several studies have shown that the frequency of β-lactam antibiotic resistance genes may vary from one hospital to another and from one country to another. Various factors such as hospital policy, sanitation measures, and medical staff can affect the prevalence of bacterial resistance genes from one bacterium to another [38–40].

K. oxytoca isolates from patients receiving antibiotic therapy and from half of the healthy individuals have the ability to produce cytotoxin. Cytotoxin exerts a damaging effect on a variety of epithelial cell culture classes such as Hep-2, Vero, Chinese hamster ovary, HeLa, and cells from rabbit colon [4,9,23].

In this study, the 23 strains (58%) identified out of 40 *K. oxytoca* isolates and the upper limit of activity assay indicated the clinical significance of this bacterium and its role in intestinal complications. It is worth noting that the majority of patients included in group A^+D^+ had diarrhoea following antibiotic use. In this study, a total of 21 children (1–15 years of age) admitted to the hospital were reviewed: seven girls and 14 boys. The cytotoxin activity in this age group was much higher among females (6, 86%) than

among males (7, 50%). In other words, the *K. oxytoca* isolates among females showed greater cytotoxin activity, although no significant relationship was found between cytotoxin activities based on the age and sex of patients. The cytotoxin activity in this age group was of high and medium levels, and a low level of activity was not observed. Out of 21 *K. oxytoca* strains isolated from children, 13 (62%) strains showed a high level of cytotoxin activity. Among the 15–75-year age group, the highest rate was noted for the male gender. Cytotoxin activity was much higher among females (5, 56%) than among males (5, 50%). There was no significant relationship between the cytotoxin activities based on the age and sex of patients. Out of 19 patients ranging in age from >15 to 75 years, 10 (53%) showed a high level of *K. oxytoca* cytotoxin activity. A study in 2010 described the role of *K. oxytoca* in the development of antibiotic-induced haemorrhagic colitis in children [8].

We can conclude that *K. oxytoca* was more prevalent in colitis caused by antibiotics in children, and that the *K. oxytoca* strains isolated from hospitalised children showed higher cytotoxin activity. Although *K. oxytoca* is considered a normal flora in the stool, it can be described as one of the causes of antibiotic-induced colitis due the action of its cytotoxin. These patients have a better chance of recovery when the treatment is discontinued the normal intestinal flora is allowed to be established or when appropriate medication is prescribed after antibiogram.

Conflict of interest

The authors declare that there was no conflicts of interest.

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